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NOTES

Evaluation of the p-Nitro-α-Acetylamino-β-Hydroxypropiophenone Differential Test for Identification of *Mycobacterium tuberculosis* Complex

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The p-nitro-α-acetylamino-β-hydroxypropiophenone (NAP) differential test for the identification of *Mycobacterium tuberculosis* recovered from clinical specimens was evaluated by two laboratories and found to be a rapid and accurate procedure with a specificity exceeding 99%.

Rapid detection of mycobacteria in clinical specimens by radiometric culture methods has been studied extensively (4, 6). However, these detection procedures only detect growth and do not differentiate between species of mycobacteria. A method for differentiating the *Mycobacterium tuberculosis* (TB) complex (*M. tuberculosis*, *M. bovis*, and *M. africanum*) from other species has been developed (2). This method is based on the selective growth inhibition of the TB complex in the presence of p-nitro-α-acetylamino-β-hydroxypropiophenone (NAP). Laszlo and Siddiqi (3) published their retrospective experience with a radiometric NAP test for 416 mycobacterial cultures: 217 clinical isolates of *M. tuberculosis* and 199 reference strains belonging to 40 mycobacterial species. They found 100% agreement with the NAP test compared to conventional biochemical testing for the differentiation of the TB complex from other mycobacteria. In our evaluation, tests were conducted by two independent laboratories to determine the accuracy of the NAP differentiation test for identifying the TB complex recovered from clinical specimens.

A total of 400 clinical isolates of mycobacteria were tested by either an indirect or a direct NAP test procedure. A total of 200 isolates (100 *M. tuberculosis* and 100 mycobacteria other than *M. tuberculosis*) obtained from the stock culture collection of patient isolates of the clinical laboratory at the University of Kentucky Medical Center were tested by the indirect test. A total of 163 isolates obtained from stock cultures of patient isolates at the Mayo Clinic were tested by the indirect method (58 *M. tuberculosis*). Isolates were also identified by conventional culture methods (1, 5). Each isolate was inoculated onto fresh Middlebrook 7H10 medium and coded so that the identification was unknown to the investigator. Suspensions were made in albumin diluting fluid to a McFarland no. 1 standard, and then rough colony types were diluted 1:10 and smooth colony types were diluted 1:100 and inoculated (0.1 ml) into a Middlebrook 12A BACTEC test vial (original vial). These bottles were incubated at 37°C for 1 to 3 days or until the growth index (GI) reached 50; then 1 ml of medium was removed and added to an NAP test vial containing 5 μg of NAP per ml. GI readings were taken daily for a maximum of 5 days from the NAP test vial and compared with normal growth in the control vial. The TB complex is inhibited in the presence of NAP and other species of mycobacteria are not, resulting in a decrease or no significant increase in GI as compared with the control vial, in which the GI increased.

An additional 37 clinical isolates recovered in the Mayo Clinic Mycobacteriology Laboratory were tested by the direct NAP test. In the direct method, when the BACTEC culture vial containing a specimen from a patient registered a GI of 50 or greater, a small volume of the medium was removed and stained for acid-fast bacilli by the auramine-rhodamine staining technique. If the acid-fast bacillus stain was positive, the specimen was checked for bacterial contamination by subculturing to a blood agar plate and observing for growth after 18 h of incubation. A 1-ml sample of culture medium from a noncontaminated test vial with a GI between 50 and 100 was directly transferred into an NAP test vial. If the BACTEC culture vial initially had a GI of 100 or greater, the culture was diluted appropriately before the NAP test was performed (product information, Johnston Laboratories, Inc., Towson, Md.). GI readings were taken daily for a maximum of 5 days on the NAP and control vials. Data were interpreted as previously stated for the indirect test method.

A total of 363 isolates of mycobacteria representing 13 species were tested by the indirect method, and 37 isolates representing 4 species were tested by the direct NAP differentiation test (Table 1). The NAP test correctly classified 399 of the 400 isolates (99.8%), with only 1 isolate of *M. tuberculosis* incorrectly classified in the indirect NAP test.

This evaluation showed that the NAP differential procedure is a reliable method for identifying *M. tuberculosis*. The number of *M. bovis* isolates available was not sufficient to assess the reliability of the test for identifying this organism. The specificity of the test exceeded 99% for the 400 clinical isolates tested. Once adequate growth is obtained for inoculation into the NAP test vial, test results are available within 5 days. In the indirect test, 13 of 163 isolates (8%) required more than 5 days to reach a GI of 50, the GI

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TABLE 1. Comparison of the NAP differential test and conventional biochemical testing in the classification of clinical isolates of mycobacterial species

NAP test used (conventional identification)	No. of strains identified[a]		% Agreement with conventional identification
	A	B	
Indirect			
M. tuberculosis	58	99	99.3
M. bovis		1	100
M. avium-intracellulare complex	20	26	100
M. kansasii	19	10	100
M. gordonae	17	36	100
M. fortuitum	16	11	100
M. chelonei	11	4	100
M. scrofulaceum	7	4	100
M. gastri	6		100
M. szulgai	5	1	100
M. xenopi	3		100
M. terrae complex	1	6	100
M. marinum		2	100
Direct			
M. tuberculosis	18		100
M. gordonae	16		100
M. avium-intracellulare complex	2		100
M. kansasii	1		100

[a] A indicates strains identified by institution A; B indicates strains identified by institution B.

necessary for NAP test vial inoculation, and delayed test results considerably.

One institution encountered difficulty in interpreting test results for *M. kansasii*. Of 19 isolates of *M. kansasii*, 5 (26%) grew particularly slowly in the NAP test vial, which led to problems with interpretation. Therefore, it is suggested that if a growth index of 5 to 10 is obtained after 5 days of testing, the bottles should be read for an additional 2 days.

With the rapid growth detection of mycobacteria and susceptibility testing of *M. tuberculosis*, the time required to report results to the physician has been reduced (6). The average times for detection of *M. tuberculosis* and mycobacteria other than *M. tuberculosis* by the radiometric system are 16 and 10 days, respectively, compared with 20 and 16 days, respectively, by conventional methods. Also, the average time to test indirect susceptibility on *M. tuberculosis* is 5 days for the radiometric method compared with at least 14 days for the conventional 1% proportion method. With the development of the NAP procedure, which characterizes mycobacteria in the TB complex, the BACTEC 460 system is capable of providing growth detection, identification, and susceptibility results in an average time of 21 days. Therefore, the addition of the NAP test to the radiometric culture and susceptibility-testing procedures provides the physician with accurate and clinically significant results considerably faster than do conventional methods of growth detection and biochemical testing. Currently, all cultures should be subcultured to conventional media so that biochemical tests can confirm the identification. This should not delay the initial report but would be important in those rare instances when *M. bovis* or *M. bovis* BCG are identified.

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